JC14 Rec'd PCT/PTO 14 DEC 2001

FORM PTO-1390 (REV 10-95) U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. §371	MERCK 2336
	U.S. APPLICATION NO. (If known, see 37 CFR §1.5) 10/018084

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP00/05205	6 JUNE 2000	16 JUNE 1999

TITLE OF INVENTION

MINIATURIZED ANALYTICAL SYSTEM WITH A DEVICE FOR WITHDRAWING SUBSTANCES

APPLICANT(S) FOR DO/EO/US

EISENBEISS, Friedhelm, et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ■ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. §371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. §371.
3. ☐ This express request to begin national examination procedures (35 U.S.C. §371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. §371(b) and PCT Articles 22 and 39(1).
4. ■ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ■ A copy of the International Application as filed (35 U.S.C. §371(c)(2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ■ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ■ A translation of the International Application into English (35 U.S.C. §371(c)(2)).
7. ■ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. §371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ■ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. §371(c)(3)).
9. ■ An oath or declaration of the inventor(s) (35 U.S.C. §371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. §371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 C.F.R. §§1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 C.F.R. §§3.28 and 3.31 is included.
13. ■ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☐ Other items or information:

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U.S. APPLICATION NO. (if known, see 37 CFR §1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
10/018084	PCT/EP00/05205	MERCK 2336

17. ☒ The following fees are submitted:

	CALCULATIONS	PTO USE ONLY
BASIC NATIONAL FEE (37 CFR §1.492 (a) (1) - (5)):		
Search Report has been prepared by the EPO or JPO $890.00		
International preliminary examination fee paid to USPTO (37 CFR §1.482) $710.00		
No international preliminary examination fee paid to USPTO (37 CFR §1.482) but international search fee paid to USPTO (37 CFR §1.445(a)(2)) $740.00		
Neither international preliminary examination fee (37 CFR §1.482) nor international search fee (37 CFR §1.445(a)(2)) paid to USPTO $1040.00		
International preliminary examination fee paid to USPTO (37 CFR §1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) $100.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$890.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 C.F.R. §1.492(e)).		

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	3 - 20 =	0	x $ 18.00	$0.00	
Independent claims	1 - 3 =	0	x $ 84.00	$0.00	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $ 280.00		
TOTAL OF ABOVE CALCULATIONS =				$890.00	
Reduction of 1/2 for filing by small entity, if applicable. A Verified Small Entity Statement must also be					
SUBTOTAL =				$890.00	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 C.F.R. §1.492(f)).					
TOTAL NATIONAL FEE =				$890.00	
Fee for recording the enclosed assignment (37 C.F.R. §1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 C.F.R. §§3.28, 3.31). $40.00 per property.					
TOTAL FEES ENCLOSED =				$890.00	
				Amount to be refunded:	
				charged:	

a. ■ A check in the amount of $890.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. 13-3402 in the amount of $ ________ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ■ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 13-3402. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 C.F.R. §§1.494 or 1.495 has not been met, a petition to revive (37 C.F.R. §1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO: Customer Number 23,599

23599
PATENT TRADEMARK OFFICE

For
SIGNATURE

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NAME

27,969
REGISTRATION NUMBER

Filed: 14 DECEMBER 2001
AJZ:kmo

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APPLICATION DATA SHEET

APPLICATION INFORMATION

Application Type::	REGULAR
Subject Matter::	UTILITY
CD-ROM or CD-R?::	NONE
Title::	MINIATURIZED ANALYTICAL SYSTEM WITH A DEVICE FOR WITHDRAWING SUBSTANCES
Attorney Docket Number::	MERCK 2336

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100180843.050302

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CORRESPONDENCE INFORMATION

Correspondence Customer Number:: 23599

REPRESENTATIVE INFORMATION

Representative Customer Number:: 23599

DOMESTIC PRIORITY INFORMATION

Application::	Continuity Type::	Parent Application::	Parent Filing Date::
This Application	National Stage of	PCT/EP00/05205	06/06/00

FOREIGN PRIORITY INFORMATION

Application Number:	Country::	Filing Date::	Priority Claimed::
199 27 535.1	Germany	06/16/99	YES

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IN THE UNITED STATES DESIGNATED/ELECTED OFFICE

International Application No.	:	PCT/EP00/05205
International Filing Date	:	6 JUNE 2000
Priority Date(s) Claimed	:	16 JUNE 1999
Applicant(s) (DO/EO/US)	:	EISENBEISS, Friedhelm, et al.

Title: MINIATURIZED ANALYTICAL SYSTEM WITH A DEVICE FOR WITHDRAWING SUBSTANCES

PRELIMINARY AMENDMENT

10018084 .050302

Commissioner for Patents
Washington, D.C. 20231

SIR:

Prior to calculating the national fee, and prior to examination in the National Phase of the above-identified International application, please amend as follows:

IN THE CLAIMS:

3. (Amended) Use of an apparatus according to Claim 1 in a planar microstructured analytical system.

REMARKS

The purpose of this Preliminary Amendment is to eliminate multiple dependent claims in order to avoid the additional fee. Applicants reserve the right to reintroduce claims to canceled combined subject matter.

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached pages are captioned "**Version With Markings to Show Changes Made**".

Respectfully submitted,

For Reg. No. 37,432

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FILED: 14 DECEMBER 2001

<u>VERSION WITH MARKINGS TO SHOW CHANGES MADE:</u>

Claim 3 has been amended as follows:

3. <u>(Amended)</u> Use of an apparatus according to Claim 1 ~~or 2~~ in a planar microstructured analytical system.

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Miniaturized analytical system having an apparatus for discharging substances

The invention relates to an apparatus for discharging defined fractions of samples after a preparative or analytical liquid-phase separation in planar miniaturized analytical systems from the separation channel into a further channel or into a further analytical apparatus.

Planar miniaturized analytical systems consist of components having incorporated channels in which the transport and/or separation of dissolved analytes is performed, for example, using capillary electrophoresis or isotachophoresis. A channel system of this type can have Y-shaped branches and/or X-shaped junctions (see Figure 1). The angles between the channels can be freely chosen in this case.

For the introduction of sample material, usually, an X-shaped arrangement of the channels is used, and for discharge a Y-shaped branch. The sample constituents for this purpose are transported electrokinetically by applying a voltage to the ends of the channels. In the case of Y-branched channels the electrokinetic transport, for example, can be diverted if the electric potentials are switched from the one channel to the other branching channel. In this manner a fraction of a sample can be discharged through the branching channel.

Such discharge operations are controlled either by time definition or actively in the case of the previous continuous-flow analysis. Time-controlled discharge requires both the exact knowledge of the electropherogram and also exact reproducibility of the separation process. The analyte to be isolated can therefore be discharged only from a known sample after previous experimental determination of its separation time. Especially for capillary electrophoresis, this

method is unsuitable, since deposition of surface-active substances causes a change in the zeta potential on the channel walls. As a result the electroosmotic force is modulated and thus also the time pattern of the electropherogram. For this reason, time-controlled discharge is usually carried out only for monitoring or verification.

Discharge of the analytes after a preceding direct analysis is considerably more accurate. F. von Heeren et al. (Anal. Chem. 68(13) (1996), 2044-2053) describe the actively controlled discharge of sodium fluorescein. The position of fluorescein during the separation process can be followed continuously by an observer using a suitable optical apparatus. As soon as the fluorescein band is situated at the discharge position, a switching operation is triggered manually which leads to discharge. However, even when this system is automated, only coloured or fluorescing substances could be detected and discharged specifically. This is a considerable restriction.

Other detector systems for discharge apparatuses have not been able hitherto to be integrated directly into miniaturized planar analytical systems, so that substances are generally detected and separated after their exit from the analytical system.

The object of the present invention, therefore, is to provide, for miniaturized planar analytical systems, an apparatus for discharging substances which is integrated directly into the analytical systems and can be controlled actively. Preferably, the apparatus for discharge should be able to be combined with detection apparatuses which are based on differing principles; this would make the discharge of analytes versatile in application.

For planar apparatuses for electrophoretic separation methods, an arrangement has been found comprising at least three transport electrodes, one detection apparatus and a switching apparatus which permits fractions to be discharged specifically during the separation operation. In preferred embodiments, the detection apparatus is constructed as an electrical conductivity apparatus, impedance apparatus or potential-measuring apparatus.

The invention therefore relates to an apparatus for discharging fractions of a sample for planar microstructured analytical systems which essentially consists of a channel system having at least one Y-branch, at least three transport electrodes and at least one detection apparatus upstream of the said branching point of the channel system and an electrical switching apparatus.

A preferred embodiment of the invention is an apparatus for discharge in which the detection apparatus is an electrochemical detector.

The present invention further relates to the use of an apparatus according to the invention in a planar microstructured analytical system.

Figure 1 shows by way of example an X-junction (X) and a Y-branch (Y) of a channel system corresponding to the prior art.

Figure 2 illustrates the principle of discharge using the inventive apparatus.

Figures 3 and 4 show diagrammatically analytical systems into which an inventive apparatus for discharging substances is integrated.

Analytical systems into which an inventive apparatus for discharging substances can be integrated are planar microstructured systems which serve for separating substances. Such predominantly two-dimensional analytical systems, due to their small size and simple fabrication, offer many advantages compared with macroscopic analytical systems. The analytical systems can comprise additional analytical apparatuses or apparatuses for micropreparative derivatization. Because of the possibility of integrating detectors or an inventive apparatus for discharge directly into these systems as early as the fabrication of analytical systems, substances can be analysed and separated even during or after separation in the analytical system. For example, more than one inventive apparatus for discharge can also be integrated into analytical systems in which substances are not only separated and analysed but are also subjected to other steps, for example derivatization steps.

The inventive apparatus for discharge consists of a detector system which analyses a channel segment directly upstream of the discharge channel, and a branched channel system having corresponding transport electrodes. As soon as the detector indicates that the desired analyte is just before the branch, the transport electrodes at the ends of the channels are switched over. Further transport is no longer carried out along the separation channel but into the branching discharge channel. This operation is terminated when the detector indicates that the analyte band has passed the discharge position. In this manner defined parts of a sample can be separated precisely from the remainder of the sample.

The discharge operation therefore comprises the following steps:

- The spatially separated sample is transported to a branch point via an appropriate electrical voltage.

- A detector which is situated closely before the branch point measures the components flowing past or a marker substance which in each case characterizes the beginning and the end of a region to be discharged.
- As soon as the desired component is detected, the voltages are switched over so that the flow is diverted into the branching channel.
- After the desired component has passed the detector, the voltage is switched back.

In this manner part of the sample is then separated spatially from the remainder of the sample.

The entire operation, particularly switching over the potentials between the electrodes, is preferably controlled by means of an electronic switching apparatus. Apparatuses of this type and their use are known to those skilled in the art. The substances separated off can then be subjected to steps leading further within the analytical system, such as separate analyses, derivatization etc. In addition they can also be specifically removed from the channel system of the analytical system. For this purpose the channel system is provided with additional outlets. These outlets are preferably situated in the discharge channels and are sealed by means of a fluidics connection, such as a tightly sealing pump or pumps and valves. Typically, a capillary follows directly, via which the fraction separated off can be transferred to further containers or instruments outside the analytical system. If therefore a separated fraction is situated in a discharge channel, it can be removed from the analytical system via an outlet hydromechanically, for example electroosmotically, or by means of micropumps.

The discharged fraction can be either an interfering constituent which is to be removed, so that the remainder of the sample can be further analysed, or else a fraction, that is to say a part of the sample,

which is to be subjected to further analytical steps or derivatization steps separately from the remainder of the sample. The sample constituents which are thus to be analysed and separated can be present in predominantly aqueous solution, ionically dissolved, emulsified, suspended, in colloidal or biological cellular form.

The basis of the inventive apparatus is a detector situated on the two-dimensional analytical system. Since miniaturized analytical systems, depending on their specific application, can have very different structures, the detector must be able to be positioned at any desired points of the analytical system. For the inventive apparatus, not only can detectors be used whose most critical parts can be integrated, such as

- conductivity detectors (capacitive, inductive, resistance measurement),
- electrochemical detectors (for example amperometry, ISFET),
- electrical temperature measurement,

but also external detectors can be used in which only parts, for example lenses or fibre optics, are integrated into the analytical system, such as

- optical detectors (for example refractive index, temperature, absorption, fluorescence, Rahman spectroscopy, luminescence)
- NMR
- radioactive labelling
- magnetic labelling

According to the invention, preferably, conductivity detectors and optical detectors are used as detector apparatus. For optical detectors, for example, a receiving apparatus for fibre optic guides can be integrated.

For analytical systems in which substances are separated electrophoretically, the requirement for a universal detection method is met particularly well by

electrical detection methods such as conductivity measurement. The analytes are characterized in this case by their specific electrical conductivity. A defined substance, in a given electrolyte system, always generates the same relative conductivity. This applies to sequential measurements in a miniaturized analytical system and also to measurements which are carried out in a plurality of miniaturized analytical systems of one type.

Preferably, in the inventive apparatus, therefore, an electrical conductivity measurement is used which, in the case of directly contacting electrodes, determines the electrical current or the electrical voltage drop or, in the case of galvanically decoupled electrodes, via measurement of the dielectric resistance.

To integrate a conductivity detector in a two-dimensional analytical system, conductivity electrodes must be integrated along the channel system at any desired points of the system, especially just before branches. This is possible only by a particular type of structure of such a system. Firstly, the channel system must be sealed gas-tightly and liquid-tightly, secondly, it must be ensured that chemically inert electrodes can be brought precisely and reproducibly to the desired positions. The abovementioned requirements can be fulfilled only by special methods for fabricating two-dimensional analytical systems.

Microfluidic or microstructured analytical systems generally consist of a continuous-flow unit which has at least the channel system and, optionally, recesses for integrating peripheral devices, and peripheral devices such as detectors, fluidic connections, reservoirs, reaction chambers, pumps, control apparatuses, etc., which can be integrated into the continuous-flow unit or connected thereto. Continuous-flow units for microfluidic analytical systems having

apparatuses for measuring and controlling electrical conductivity are inventive systems in which, by joining together at least two components, for example substrate and cover, microchannel structures are produced which can be sealed liquid-tightly and/or gas-tightly.

The inventive systems typically consist of at least two components, a cover, which is provided with the electrodes, and a microstructured substrate. After production of the components, these are joined together by a special bonding process. In this manner it is possible to integrate the inventive discharge apparatus into planar analytical systems.

The components of the continuous-flow unit of the analytical systems preferably consist of commercially available thermoplastics such as PMMA (polymethyl methacrylate), PC (polycarbonate), polystyrene or PMP (polymethylpentene), cycloolefinic copolymers or thermosetting plastics, for example epoxy resins. More preferably, all components of a system consist of the same material.

The components can be produced by methods known to those skilled in the art. Components which comprise microstructures can be produced, for example, by established processes, such as hot embossing, injection moulding or reaction casting. Particular preference is given to the use of components which can be duplicated by known methods for mass production. Microstructured components can have channel structures of cross sectional regions between 10 and 250 000 μm^2. For the inventive discharge apparatus, the channel system must have, in addition to regions for sample feed and a separation channel, at least one X- or Y-branch departing from a separation channel. For the integration of a plurality of discharge apparatuses, further branches can be introduced at any desired points of the channel system.

The electrodes which are required for the inventive discharge apparatus are transport electrodes which are situated at the ends of the branched channels and make it possible to switch over the potential between the two channels, and detection electrodes which are preferably positioned between 40 mm and 0.1 μm upstream of the branch.

For the integration of the electrodes into the analytical system or the inventive apparatus, the electrodes are preferably mounted on one component of the system, the cover. For this purpose they must have a sufficient strength of adhesion to the plastic component. This is of importance both for joining the individual components together and also for the later use of the analytical systems. If adhesives, for example, are used in the bonding of the components, the adhesive must not detach the electrodes from the plastic surface. In addition, the electrodes should consist of chemically inert materials, for example noble metals (platinum, gold).

Plastic surfaces are typically metallized by electrochemical deposition of metals from metal salt solutions. For this it is generally customary, in a multistep process, first to pretreat the plastic surface chemically or mechanically, to apply a discontinuous primer and then to carry out the electrochemical deposition. Descriptions of these metallization techniques may be found, for example, in US 4,590,115, EP 0 414 097, EP 0 417 037 and in Wolf and Gieseke (G.D. Wolf, H. Gieseke, "Neues Verfahren zur ganzflächigen und partiellen Metallisierung von Kunststoffen" [Novel Process for Complete and Partial Metallization of Plastics], *Galvanotechnik* 84, 2218-2226, 1993). All of the wet-chemical processes share the fact that relatively complex pretreatment processes are necessary to achieve sufficient adhesion strengths.

DE 196 02 659 describes the application of copper with strong adhesion strength to multiphase polymer blends using vaporization or sputtering. The reason mentioned for the good adhesion is the composition of the polymer blends. According to this the blends must comprise polyarylene sulphides, polyimides or an aromatic polyester.

The effect of plasma pretreatments to achieve improved adhesion properties of metals on plastic surfaces is summarized by Friedrich (J. Friedrich, "Plasmabehandlung von Polymeren" [Plasma Treatment of Polymers], *kleben & dichten* 41, 28-33, 1997) for the example of various commercially available thermoplastics.

Particularly preferably, the electrode structures are produced on the plastic components using a two-layer method. For this purpose, first an adhesion-promoting layer of chromium oxide is produced. In contrast to noble metals, chromium oxide displays outstanding adhesion properties on plastic surfaces. In addition, chromium oxide, in contrast to elemental chromium and other transition metals, is considerably more resistant to redox processes. The noble metal, for example platinum or gold, or alloys of these metals, is then applied to the adhesion layer of chromium oxide.

Chromium oxide and the noble metal layer to be deposited thereon are preferably selectively applied to plastic substrates in the lift-off process or by means of the shadow-mask method or the structuring of metal layers initially applied on whole surfaces. These operations are standard processes in microstructuring. Below, the working steps necessary for the two-layer method are briefly described for the said processes.

Lift-off process: the plastic component to be metallized selectively is coated with a photoresist. This photoresist must not etch the plastic part to be metallized, or etch it only slightly. For PMMA, for example, a photoresist from Allresist, Berlin (AR 5300/8) has proved to be suitable. After exposure and development of the structures to be metallized, the metal layers are applied in a sputtering unit. The chromium oxide layer is applied during the sputtering process by introducing oxygen into the sputtering unit argon plasma typically used. The sputtering target used is a conventional chromium target. Typical chromium oxide layer thicknesses are 10-50 nm. Alternatively, a chromium oxide target can be used directly. Platinum or its alloys or gold is then sputtered directly under standard conditions, i.e. in the argon plasma. It has also proved to be advantageous for the adhesion strength of the chromium oxide layer to backsputter the plastic in an oxygen/argon (approximately 5% by volume/95% by volume) plasma before sputtering the chromium oxide. In the actual lift-off process, the photoresist still present, and together with this the metal layer situated on the resist, are detached from the plastic component in a developer from Allresist (AR 300-26).

Shadow-mask method: the plastic part to be metallized selectively is covered with what is termed a shadow mask. This has openings at the regions to be metallized. The metal layers are sputtered through these similarly to the lift-off process.

Structuring planar metal layers: a metal layer is firstly applied, similarly to the above-described sputtering process, over the whole surface of a plastic part to be metallized selectively. This metal layer is structured in subsequent process steps, either by selective erosion using, for example, laser ablation (gold and platinum) or, for example, by selective wet-

chemical etching. For structuring using wet-chemical etching, a photoresist (Hoechst AG, Germany; AZ 5214) is first applied to the metal layer, exposed and developed. Gold is then removed in the exposed regions in cyanide solution. The non-electrically-conducting chromium oxide layer remains behind. The remaining photoresist is then removed with a developer (for example AR 300-26, Allresist, Berlin).

The adhesion strength of electrodes produced with chromium and also chromium oxide as adhesion layer using the sputtering method has been tested using tear-off tests. The adhesion strength of the chromium oxide layers is significantly greater. The metal layers that were produced using chromium oxide as adhesion layer are also markedly more resistant in ultrasonic treatment in alkaline solution compared with metal layers that were produced using chromium as adhesion layer.

After production and preparation of the individual components, these are joined together by the process of the invention. Preferably, one component, the substrate, is microstructured and provided with rear-side bore holes or recesses for filling the channels and/or contacting the electrodes. In addition, the use of a so-called sealing lip, that is to say an elevation on the substrates which completely encloses the channel structures between typically 0.5 and 5 µm in height has also proved to be highly advantageous with respect to the adhesion process. The other component, the cover, serves for covering and, for example, is provided with the electrodes in the case of electrophoretic analytical systems. In this case the cover according to the invention is termed electrode cover. For certain applications, the analytical systems can require a functionalization of the components deviating from this preferred arrangement. In this case, for example, more than two components, for example two covers and one

substrate etc., can be joined together in order to generate channel structures which lie one above the other, or other functionalities, such as detection systems, reaction chambers etc., can be integrated into the components. According to the invention, all parts of the continuous-flow unit of the analytical system which are joined together using a bonding process are termed components. They can be microstructured, provided with electrodes or have other functionalities. A subdivision of the components into substrates and covers or electrode covers, if the respective component is provided with electrodes, only serves for the more detailed description of the embodiment of the specific components and does not represent any restriction with respect to other properties of the components, such as microstructuring etc., or their combinations with one another.

The components are joined together according to the invention with high precision. The adhesive must not run into the channels and coat their surfaces, since this can alter the surface properties of the channels. It has been found that this leads, for example, to increased adhesion of analytes, for example proteins, in the channel regions which are wetted with adhesive. This in turn affects the separation quality of the analytical systems. Similarly, sticking of adhesive to the electrodes impairs their functionality.

It is also of great importance that the volume of the channels is not changed, as would occur, for example, by the uncontrolled ingress of adhesive. According to the invention the channel, to improve the sensitivity of detection, is preferably constricted in the vicinity of the detection electrodes. As a result it is important precisely in these regions that no adhesive passes into the channel.

To join the components together, according to the invention an adhesive is preferably applied firstly to the microstructured component at the points at which no structuring is present. The layer thickness is between 0.5 and 10 µm, preferably between 3 and 8 µm. Typically, it is applied using a flat roller application known from printing technology.

In a preferred embodiment, a thin adhesive film is applied for this purpose via a structured metallic screen roll which takes up a defined volume of adhesive, to a second unstructured roll which is coated with a polymer. From this, in turn, application is performed directly onto the structured substrate in such a manner so as preferably to give an adhesive thickness between 3 and 8 µm on the unstructured surface of the substrate. Depending on the plastic (substrate material) used, the transfer between the plastic roll and the substrate is influenced by any viscosity increase in the adhesive (prepolymerization). An important advantage of this process is that the substrate need not be positioned relative to the roll applying the adhesive and nevertheless adhesive is only applied in the unstructured regions of the substrate. If too much adhesive is applied, when the cover and substrate are pressed together, adhesive will flow into the channel. If in parts insufficient adhesive has been applied, leaks in the channel structure result. This bonding method requires a flatness of the components of preferably less than approximately 5 µm/cm of component length.

The adhesive used must not etch the surface of the components or etch it only slightly, in order that the electrodes during the adhesion process are not detached or interrupted by the adhesive. Preferably, therefore, the adhesive used is the product NOA 72, thiolacrylate from Norland, New Brunswick NJ, USA. This glue is cured photochemically. However, other types of glues can also

be used for the process, for example thermally curing glues which comply with the abovementioned conditions.

After the adhesive has been applied, the second component having the thin-layer electrodes is positioned appropriately to the substrate for example on an exposure machine and pressed on. For this purpose, preferably the substrate together with the applied adhesive is fixed in the exposure machine in the position otherwise provided for silicon wafers. Preference is given to the use of thick glass plates as pressing surface, since in this manner the positioning and photochemical curing of the glue can be carried out directly by illumination with an Hg lamp (emission wavelength 366 nm). The electrode cover is fixed in the position provided for the exposure mask by holding it with a vacuum apparatus milled in a glass plate. Since both the electrode cover and the glass plate used for holding the cover are transparent, the cover can be aligned with respect to the substrate through this arrangement. If the cover extends beyond the substrate, this cover can also be held mechanically.

For the adhesion process, typically in addition to optomechanical alignment with the assistance of optical alignment marks, the cover can also be positioned on the substrate passively mechanically using a push-in apparatus, optomechanically without special alignment marks, or electromechanically using electrical marks (contacts).

It has been found that the preferred optical metallic alignment marks on the cover can be applied together with the electrodes in the same process step, that is to say preferably can be sputtered, that is to say no additional expenditure is necessary. The corresponding counterstructures on the substrate also require no additional processing, since these are introduced into the substrate together with the channel structures in a

moulding step. For the optomechanical alignment, at least one component must consist of a transparent plastic. Using the inventively applied alignment marks, the two components are positioned with respect to one another at an accuracy of at least ± 10 µm, typically even ± 2 µm (for example of theoretical to actual position of the detector electrode) and pressed together. The high position accuracy supports the achievement of reproducible analytical results. The adhesive is then polymerized by a UV lamp. After turning off the vacuum for holding the cover or releasing the mechanical fixing the continuous-flow unit is removed from the exposure machine.

In another preferred embodiment, a component is provided with adhesive by means of a process known in printing (pad printing). The component provided with the electrodes is wetted with the glue for this purpose on the regions which, when the two components are combined, must not lie over a channel or be electrically contacted. Microstructured components are wetted such that no adhesive passes into the channel structure or other recesses. Pad printing is a structured glue application. Adhesive is stored in a negative mould of the substrate. Via a typically silicone pad, this adhesive is taken up in a structured manner and applied, for example, to the cover in such a manner that the regions which later form a wall of a fluidic channel are not wetted with adhesive. The component having the channel structures is then, as described above, positioned in a suitable manner to its counterpiece and pressed on. Curing is performed as described above.

A structured glue application using spray methods (for example microdrop methods) or using screen-printing methods is also possible, provided that the lateral resolution of the glue discharge is sufficient.

Pressing on the second component or pressing together the components, for the purposes of the invention, means that the components are suitably brought into contact with one another. In order to achieve, after curing, a permanent bonding of the components, it is generally not necessary to exert a large force, that is to say to press the components together very firmly.

If the curing process of the glue is carried out outside the alignment apparatus used for positioning the cover and substrate, the metallized cover and the substrate, after they have been aligned with one another, can be initially attached by laser welding. After this the composite is removed from the alignment apparatus and the adhesive used is cured in a separate exposure apparatus or an oven. This procedure means a process acceleration and simplification, since the curing need no longer be performed in the alignment apparatus.

Since the thermoplastic materials preferably used are very largely transparent to laser light in the visible and near-infrared wavelength range, laser welding in this wavelength range requires an absorber layer for absorbing the optical power at the interface between cover and substrate. This absorber layer is applied simultaneously with the application of the power electrodes or detector electrodes. For example, the electrode cover, during sputtering of the electrodes with noble metal, can additionally be sputtered at further points with a noble metal layer as absorber layer.

Welding an electrode cover, which is provided with 200 nm thick platinum electrodes and therefore also comprises additional platinum surfaces for absorbing the laser power, to a substrate (base material PMMA) is performed using diode laser radiation (mixture of wavelengths of 808, 940 and 980 nm) at a power of

40 watts and a focus diameter of 1.6 mm. The platinum layer is destroyed during welding.

Alternatively, it is also possible to use a substrate or cover filled, for example, with carbon black particles, as absorber. This last-mentioned procedure, however, has the disadvantage that then at least one channel wall consists of another material. The possibilities for input into the channel or output from the channel of optical power for optical detection purposes are also restricted thereby.

The contacting of the transport electrodes and detection electrodes and the automatic control and switching of the electric current are performed by methods known to those skilled in the art.

In this manner, transport and detection electrodes can be integrated into the microstructured analytical systems in such a manner that one or more inventive discharge apparatuses are produced. The integration of the discharge apparatuses neither requires additional expenditure nor affects the quality (stability, size etc.) of the analytical systems.

The inventive apparatus thus represents an important additional functionality for planar microstructured analytical systems. It enables for the first time multifunctional microstructured analytical systems and continuous-flow units for analytical systems to be designed. The systems are not only able to separate samples, but can rather separate, identify and select samples without the sample leaving the analytical system. This also opens up the possibility of passing only certain sample constituents out of the analytical system after the discharge, or subjecting these sample constituents to further derivatization steps or analytical steps in the system.

By means of the described methods for fabricating the electrodes and bonding methods, for the first time closed microchannel structures can be produced in which electrodes can be positioned at any desired points within the channels. Structured components (substrates) can be provided liquid-tightly and gas-tightly with, for example, electrode covers. By using chiefly commercially available plastics and simple processing steps, analytical systems of this type can be produced inexpensively and in great numbers. By means of the special joining or bonding process the components are wetted with adhesive in such a manner that after the joining no adhesive passes into the interior of the channel system, that is to say into the channels, the walls or onto electrodes protruding into the channel system or other apparatuses. As a result, the separation quality and analytical sensitivity of the systems is improved. They meet all requirements which must be made of an analytical system which can be variable in use and operates precisely:

- They exhibit high dimensional and volumetric stability of the channels.
- As a result of the strength of the adhesive bonds they are pressure-stable in the interior of the channels.
- There is great variety with respect to the plastics which can be used.
- Chemically inert materials can be used for components and electrodes.
- All four channel walls preferably consist of the same material.
- The electrodes can be positioned at any desired points of the channels with an accuracy of ±10 μm.
- The contact surfaces of the electrodes are free from contamination due to adhesive.
- The electrodes can easily be connected.
- The systems exhibit low internal resistance and permit potentially high current densities.

Figure 2 illustrates the discharge of a substance using the inventive apparatus. Three different stages of discharge are shown in Figures A, B and C. The diagrammatic discharge apparatus consists of a Y-branched channel system having the transport electrodes 1, 2 and 3 at the ends of the channels. The channel piece between electrodes 1 and 2 serves as separation channel, and the channel branching off towards electrode 3 is the discharge channel. Just upstream of the branch of the discharge channel is situated a detector electrode 4 in the separation channel. In Figure A, the substances 5 and 6 to be separated migrate along the separation channel owing to a potential between the electrodes 1 and 2. Figure B shows the moment at which the desired substance 5 passes the detector electrode. The detected signal, for example the specific relative conductivity, causes the potential to be switched over, so that there is then a potential between electrodes 1 and 3. As a result, as shown in Figure C, substance 5 migrates into the discharge channel and is thus separated from substance 6 which is situated in the separation channel. After substance 5 has passed the detector region and has migrated into the discharge channel, the potential can be switched over again, so that no further substances pass into the discharge channel.

Figure 3 shows diagrammatically an example of a miniaturized planar analytical system having an integrated inventive apparatus for discharging samples. The system comprises a separation channel T1 having two transport or power electrodes E3 and E5 at the ends and two detection electrodes E1 and E2 just upstream of the branching point V of the channel system. At the branching point V one channel branches off which in turn branches into three. At the ends there are situated a reservoir P, a mixing reactor R and a further reservoir having a power electrode E4. If a substance mixture is then separated along the

separation channel T1, using the detection electrodes E1 and E2, it can be established when the desired sample substance passes the branching point V of the channel system. At this moment the potential is switched over so that there is then a potential difference between E5 and E4. As a result the selected substance migrates into the branch of the channel system. After the detection electrodes E1 and E2 indicate that the substance has passed the branching point, the potential can be switched over again. The substance isolated in the branch can then be transported mechanically by a liquid stream from the reservoir at E4 into the mixing reactor R. In addition, by a similar liquid stream starting from the reservoir P, other substances, for example reactants for derivatization, can be passed in parallel into the mixing reactor, where they mix with the sample substance and, if appropriate, react with it.

Figure 4 shows diagrammatically an example of a miniaturized planar analytical system having an integrated inventive apparatus for discharging samples, an apparatus for the delivery of defined sample volumes and a separation channel. Such an analytical system offers the possibility of delivering a defined large sample volume, of fractionating this, for example, by means of ITP, of separating a defined fraction of the sample by the inventive discharge apparatus and optionally of fractionating again and analysing, or of removing from the system, the fraction separated off or the remainder of the sample. The sample delivery apparatus consists of the channel sections K1 and K2, which are bounded by the fluidic connections F1 and F2, and F2 and F3. Typically, tightly sealing micropumps or micropumps and valves serve as fluidic connections. The volume of channel section K1 is typically 5 or 10 μl, and that of channel section K2 is 0.5 or 1 μl. By opening the fluidic connection F2 and simultaneously filling the channel section K1 with the sample solution

via the fluidic connection F1, a defined sample volume, determined by the volume of K1, is charged into the channel system. A greater volume can be delivered if, instead of the fluidic connection F2, the fluidic connection F3 is opened. The volume of sample delivered is then given by the sum of the volumes of channel sections K1 and K2. If the volume of sample delivered is to be less, in contrast, by opening the fluidic connections F2 and F3, only the channel section K2 is charged. By varying the size of channel sections K1 and K2 or else by adding other channel sections bounded by fluidic connections, the delivery volume can thus be varied and matched to the corresponding sample requirements. The sample is fractionated in the following channel systems (K3, K4, K5). For this purpose, at the ends of the overall channel system, that is to say following K1, K4 and K5, there are situated in each case liquid reservoirs or buffer reservoirs R1, R2 and R3 and power electrodes L1, L2 and L3. The buffer reservoirs can be charged via the fluidic connections F1, F4 or F5. If only channel section K1 is used for sample delivery, in addition channel section K2 can be used for extending the separation path. The sample separation can be extended, for example in the case of purely analytical questions, via channel section K3 up to channel section K5. Detection is then performed using the detection electrodes D3 and D4 mounted just upstream of R3. If a fraction of the sample is to be separated from the remainder, the inventive apparatus is used for discharge. This apparatus is formed by the separation channel section K3, the branching point Vz, the two branching channel sections K4 and K5, the detection electrodes D1 and D2 which are situated upstream of the branching point Vz and by a switching apparatus, which is not shown in the drawing, for controlling the power electrodes. As soon as the desired fraction has passed the detection electrodes D1 and D2 during the separation, the potential of the power electrodes L1,

L2 and L3 can be modified appropriately. If transport from L3 to L2 was initially performed, the fraction can be discharged into the channel K4 at the branching point Vz by switching over to a potential difference between L3 and L1. After the fraction has passed Vz, the remainder of the sample is again transported in K5 by switching over again. The discharged fraction can then be taken off from the analytical system via the fluidic connection F4. The remainder of the sample remaining in K5 can be analysed again via the detection electrodes D3 and D4. Similarly, the fraction to be discharged can be discharged into channel section K5 at Vz by a different switching of the power electrodes, while the remainder of the sample is transported into channel section 4. In this case, the discharged fraction can be detected again at D3/D4. In addition, there is the possibility of charging the channel system with two different buffer systems and thus carrying out two different separations directly one after the other. For this purpose the channel sections K3 and optionally in addition K2 are filled with the first buffer system. From the branching point Vz, the channel sections K4 and K5 are charged with the second buffer system. The first separation is performed along K2/K3. At Vz, a fraction of the sample can then be discharged into channel section K5 or else all of the sample can be transferred to this channel section. As soon as the sample has reached this channel section with the other buffer system, the second separation takes place. The two separations are monitored via the detection electrodes D1 and D2 for the first separation and the optional discharge, and with D1/D2 for monitoring the second separation. In this manner, for example an isotachophoretic separation and an electrophoretic separation, or else two isotachophoretic separations, can be combined.

Even without further explanations, it is assumed that a person skilled in the art can utilize the above

description to the broadest extent. The preferred embodiments and examples are therefore to be understood only as descriptive disclosure which is in no way limiting in any sense.

Complete disclosure of all applications, patents and publications listed above and below, and of the corresponding application DE 199 27 535, submitted on 16.06.1999, is incorporated by reference into this application.

Claims

1. Apparatus for discharging fractions for planar microstructured analytical systems essentially consisting of a branched channel system, at least three transport electrodes, at least one detection apparatus upstream of a branching point of the channel system and an electrical switching apparatus.

2. Apparatus according to Claim 1, characterized in that the detection apparatus is an electrochemical detector.

3. Use of an apparatus according to Claim 1 or 2 in a planar microstructured analytical system.

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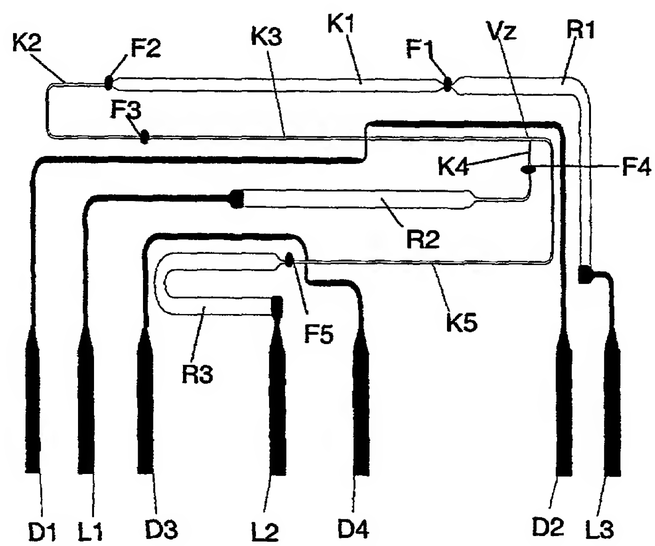
[Fortsetzung auf der nächsten Seite]

(54) Title: MINIATURIZED ANALYTICAL SYSTEM WITH A DEVICE FOR WITHDRAWING SUBSTANCES

(54) Bezeichnung: MINIATURISIERTES ANALYSENSYSTEM MIT VORRICHTUNG ZUM AUSSCHLEUSEN VON SUBSTANZEN



(57) Abstract: The invention relates to a device for withdrawing defined components of samples from the separation channel and transporting them to another channel after a preparative or analytical liquid phase separation in flat, miniaturized analytical systems. The withdrawal device can be directly integrated into the analytical system by positioning detectors, for example for measuring the conductivity, any place in the analytical system.

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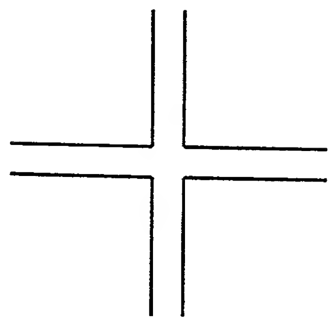
Fig. 1



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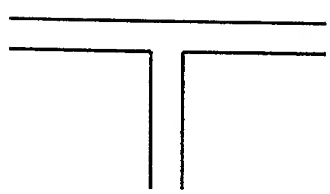

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Fig. 2

A

B

C

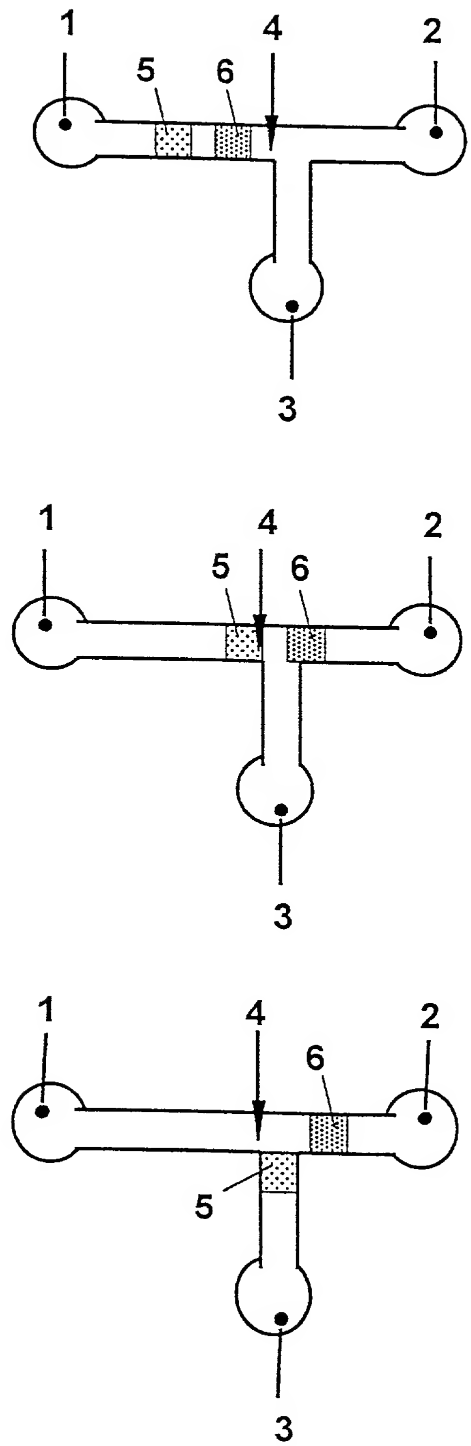

Fig. 3

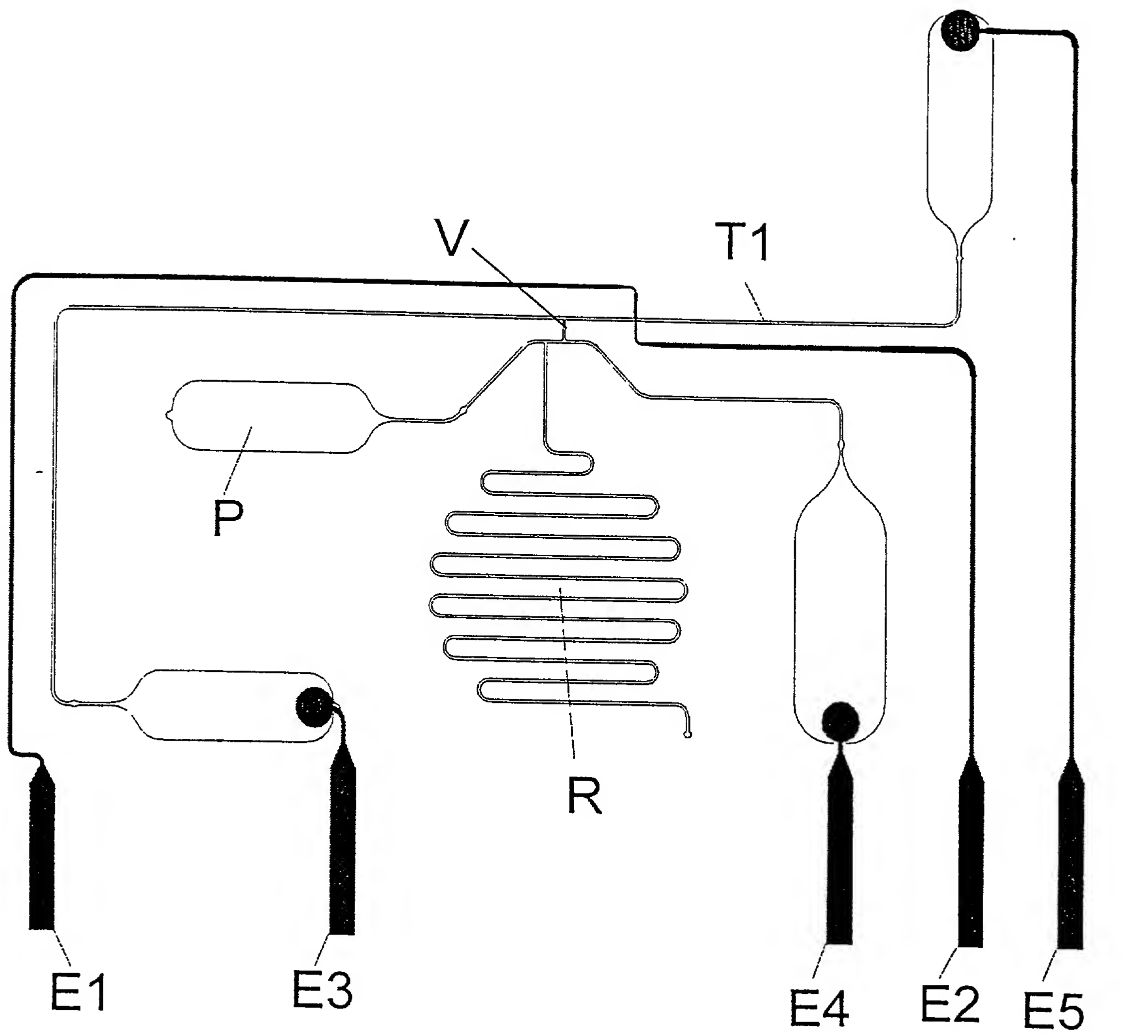

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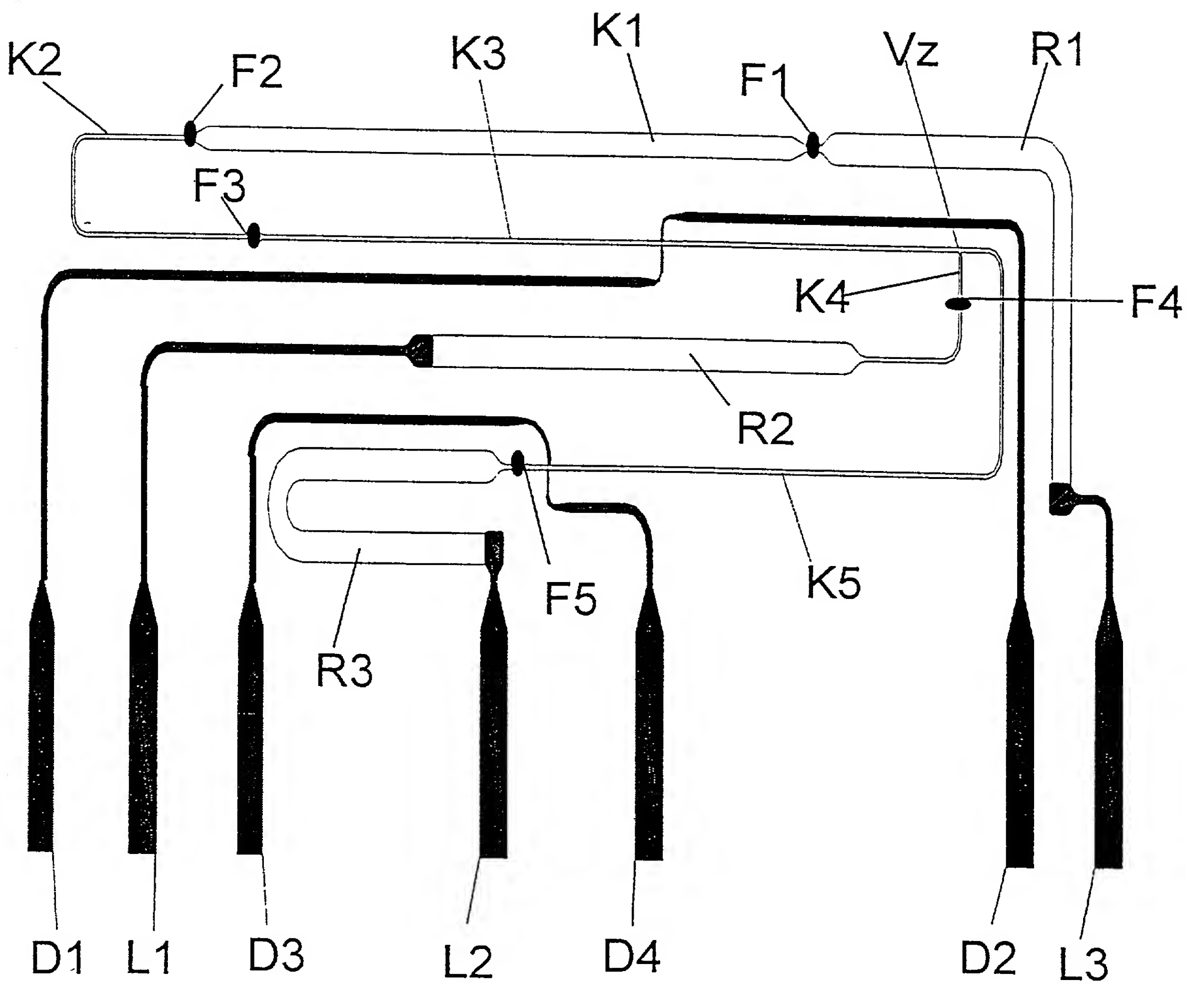
Fig. 4



COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (Includes Reference to PCT International Applications)	ATTORNEY'S DOCKET NUMBER

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Miniaturized Analytical System with a Device for Withdrawing Substances

the specification of which (check only one item below):

☐ is attached hereto.

☐ was filed as United States application

Serial No.

on _____

and was amended

on _____ (if applicable).

☒ was filed as PCT international application

Number PCT/EP00/05205

on 6. June 2000,

and was amended under PCT Article 19

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I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR § 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

I hereby claim priority benefits under Title 35, United States Code, § 119 or 365 (b) of the following United States provisional application(s) and of any foreign application(s) for patent or inventor's certificate or 365(a) of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

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COUNTRY (if PCT, indicate "PCT")	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 USC 119
Germany	199 27 535.1	16. June 1999	☒ YES ☐ NO
			☐ YES ☐ NO
			☐ YES ☐ NO
			☐ YES ☐ NO
			☐ YES ☐ NO

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Combined Declaration for Patent Application and Power of Attorney (Continued)

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	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
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SIGNATURE OF INVENTOR 206 Benedikt Beutel	16.04.2002	SIGNATURE OF INVENTOR 212	DATE